

APPENDIX H

Mycobacteriology *Lab Tests*

Quick Reference Sheets

October 2004

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- Genotyping
- Gen-Probe
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- LJ Slants
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7H11 Agar Plate

How does it work?	7H11 agar is a culture media for the isolation and cultivation of mycobacteria. Oleic acid, albumin, and pancreatic digest of casein are the key ingredients which aid in the growth of the tubercle bacilli.
When would this media be used?	When a broth culture exhibits growth, the laboratory uses this media to obtain growth of the mycobacteria on solid media.
How long before growth is obtained?	Visible growth can occur in as few as 3 to 5 days with the rapid-growing mycobacteria. With <i>M. tuberculosis</i> , and some of the other slow-growing bacteria, it can take up to 4 weeks before growth is obtained.
How are the results classified?	Positive for growth Negative for growth Contaminated
What do the results mean?	When growth is observed on the 7H11 media, the technologist determines if the growth is a mycobacterium species or if it is some other organism. If the growth is a mycobacterium species, identification procedures are started. If the growth proves to be an organism other than a mycobacterium, then the plate is considered to be contaminated and no further studies are performed. If no growth is seen on the 7H11 agar, it is reported as negative.
Are other tests needed?	The TB Lab will initiate identification procedures if the growth is a mycobacterium species.
How would this test be ordered?	NA
How much would this test cost?	The GA Public Health Laboratory does not charge the county or the district for this procedure.

Acid-Fast Stain (AFB Smear)															
How does it work?	<p>Mycobacteria are able to form stable complexes with certain stains such as Auramine O. Although the exact nature of the acid-fast staining reaction is not completely understood, phenol in the primary stain allows the stain to penetrate into the cell wall. The cell wall mycolic acids retain this primary stain even after washing with acid-alcohol. This resistance to decolorization with acid-alcohol is what causes mycobacteria to be called “acid-fast.”</p> <ul style="list-style-type: none"> • A drop of processed sputum is spread on a microscope slide and placed on a 70° C slide warmer for 2 hours. • The slide is then moved to a staining rack and Auramine O stain is applied to the slide for 15 minutes. • The slide is washed with acid-alcohol for 2 minutes. • Acridine orange, a counterstain, is applied to the slide for 2 minutes. • The slide is then rinsed with distilled water, allowed to air dry, and examined using a fluorescent microscope. <p>Mycobacteria appear as green fluorescing bacilli against a red-orange background.</p>														
When is this test run?	Monday through Friday														
How long before results are ready?	Results are usually available within 24 hours.														
How are the results classified?	<table> <tr> <th><u>Number of bacteria seen</u></th><th><u>Interpretation</u></th></tr> <tr> <td>No fluorescing bacteria seen</td><td>No AFB found</td></tr> <tr> <td>1-3 fluorescing bacteria seen on entire slide</td><td>+/-</td></tr> <tr> <td>4-36 fluorescing bacteria seen per 100 fields</td><td>1+</td></tr> <tr> <td>4-36 fluorescing bacteria seen per 10 fields</td><td>2+</td></tr> <tr> <td>4-36 fluorescing bacteria per field</td><td>3+</td></tr> <tr> <td>>36 fluorescing bacteria per field</td><td>4+</td></tr> </table>	<u>Number of bacteria seen</u>	<u>Interpretation</u>	No fluorescing bacteria seen	No AFB found	1-3 fluorescing bacteria seen on entire slide	+/-	4-36 fluorescing bacteria seen per 100 fields	1+	4-36 fluorescing bacteria seen per 10 fields	2+	4-36 fluorescing bacteria per field	3+	>36 fluorescing bacteria per field	4+
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What do the results mean?	<ul style="list-style-type: none"> • The higher the number (4+), the higher the bacterial load. • The higher the number, the more infectious the patient is to others. • Begin TB treatment with 4 drugs until TB is confirmed or ruled out. • Any patient with a positive AFB smear needs to be on respiratory isolation. 														
Are other tests needed?	Yes, a culture and sensitivity if the culture grows MTB.														
When would this test be used?	<ul style="list-style-type: none"> • To quickly determine if TB is a possibility. • To determine the degree of infectiousness. 														
How would this test be ordered?	This test is automatically done on all clinical specimens.														
How much would this test cost?	The GA Public Health Laboratory does not charge the county or district for this test.														

Genotyping	
How does it work?	<p>The genotype of an organism refers to the specific genetic makeup of that organism, usually in the DNA. This “internally coded, inheritable information” is used as a blueprint or set of instructions for building and maintaining a living creature. These instructions are written in a coded language (the genetic code) and they are copied at the time of cell division or reproduction and are passed from one generation to the next.</p> <p>Genotyping describes a great variety of techniques that are used to identify the primary localization and mapping of genes.</p>
When is this test run?	The State Lab does not perform this test. Isolates are sent to the California State Genotyping Lab for analysis.
How long before results are ready?	Results are usually available 2 to 4 weeks after the California State Genotyping Lab receives the isolate.
How are the results classified?	NA
What do the results mean?	The results show a mapping of the gene.
Are other tests needed?	NA
When would this test be used?	<ul style="list-style-type: none"> • To rule out cross-contamination of specimens. • To determine if two or more patients are infected with strains of <i>M. tuberculosis</i> possessing identical genotypes. • To determine reactivation vs. reinfection of a patient.
How would this test be ordered?	Isolates on all first-time MTB positive patients are automatically sent to Michigan for genotyping. Physicians can request this test by calling the Georgia Public Health TB Lab.
How much would this test cost?	There is no charge for this test.

Gen-Probe AccuProbe DNA Probe Test (Gen-Probe)	
How does it work?	<ul style="list-style-type: none"> The Gen-Probe identification test is a rapid DNA probe test which uses nucleic acid hybridization for the identification of certain mycobacteria. Gen-Probe manufactures four Accuprobe kits to test for the following organisms: <i>M. tuberculosis</i> complex, <i>M. avium</i> complex, <i>M. gordonae</i>, and <i>M. kansasii</i>. Ribosomal RNA is released from the test organism by sonication. A single-stranded DNA probe (specific for the target* organism) with a chemiluminescent label combines with the ribosomal RNA to form a DNA:RNA hybrid. The hybrids are then measured in a luminometer. Results are measured in relative light units.
When is this test run?	Tuesdays and Thursdays
How long before results are ready?	Results are available the day the test is run.
How are the results classified?	Positive, Negative, and Indeterminate for the target organism.
What do the results mean?	<p>Positive: The isolate is identified as the target organism.</p> <p>Indeterminate: The test is inclusive and must be repeated.</p> <p>Negative: The isolate is not the target organism.</p>
Are other tests needed?	If the isolate is identified as <i>M. tuberculosis</i> , and if this is the first time the patient has had a positive MTB culture, then a susceptibility should be ordered. This is automatically done by the TB Lab.
When would this test be used?	To identify growth on solid media or growth in liquid media as <i>M. tuberculosis</i> complex, <i>M. avium</i> complex, <i>M. kansasii</i> , or <i>M. gordonae</i> .
How would this test be ordered?	This test is ordered by the TB laboratory technologists when it is indicated.
How much would this test cost?	The GA Public Health Laboratory does not charge the county or the district for this test.

* The target organism is the organism the test kit is designed to identify. For example, if you were using the Accuprobe *M. kansasii* test kit, the target organism would be *M. kansasii*.

High-Performance Liquid Chromatography (HPLC)	
How does it work?	HPLC is used to identify mycobacteria by analysis of mycolic acids. Mycobacteria contain large amounts of mycolic acids in their cell wall. The type and amount of the mycolic acids vary in each species of mycobacteria. HPLC testing generates chromatographs based on the mycolic acids that are present in the test organism. By comparing these graphs with know reference chromatograph patterns, the identification of the organism can be determined.
When is this test run?	Fridays and Mondays. It requires 2 days to run HPLC testing. Testing is started on Fridays and finished on Mondays. The chromatographs are read on Tuesdays.
How long before results are ready?	Results (organism identification) are usually available on Tuesday.
How are the results classified?	HPLC testing should identify the mycobacterium being tested.
What do the results mean?	N/A
Are other tests needed?	If the organism is identified as M. tuberculosis, and if this is the first time MTB has been isolated from the patient, then a susceptibility needs to be ordered (done automatically by the TB Lab).
When would this test be used?	To identify an isolate suspected of being a mycobacteria.
How would this test be ordered?	This test is ordered by the TB laboratory technologists when it is indicated.
How much would this test cost?	The GA Public Health Laboratory does not charge the county or the district for this test.

Lowenstein-Jensen Agar (LJ Slants)

How does it work?	Lowenstein-Jensen agar is a relatively simple formulation that requires the addition of supplements in order to support the growth of mycobacteria. Glycerol and egg mixture are added to provide the fatty acids and protein which are required for the metabolism of mycobacteria. When this media is sterilized, the coagulation of the egg albumin provides a solid agar used for inoculation purposes.
When would this agar be used?	After undergoing the decontamination/concentration process, all specimens received in TB Lab for routine culture are inoculated onto this media.
How long before growth is obtained?	Visible growth can occur in as few as 3 to 5 days with the rapid-growing mycobacteria. With <i>M. tuberculosis</i> , and some of the other slow-growing bacteria, it can take up to 4 weeks before growth is obtained.
How are the results classified?	Positive for growth Negative for growth Contaminated
What do the results mean?	When growth is observed on the LJ slant, the technologist determines if the growth is a mycobacterium species or if it is some other organism. If the growth is a mycobacterium species, identification procedures are started. If the growth proves to be an organism other than a mycobacterium, then the LJ is considered to be contaminated and no further studies are performed. If no growth is seen on the LJ slant, it is reported as negative.
Are other tests needed?	The TB Lab will initiate identification testing. Susceptibilities will be ordered by the lab when indicated.
How would this test be ordered?	NA
How much would this test cost?	The GA Public Health Laboratory does not charge the county or the district for this procedure.

Mycobacteria Growth Indicator Tube (MGIT)

How does it work?	<p>The MGIT Tube is intended for the detection and recovery of mycobacteria using the BACTEC 960 equipment. The tubes contain 7 ml of modified Middlebrook 7H9 broth and are flushed with 10% CO₂.</p> <p>A fluorescent compound is embedded in silicone on the bottom of the round bottom MGIT tubes. The fluorescent compound is sensitive to the presence of oxygen dissolved in the broth. Initially, the large amount of dissolved oxygen quenches emissions from the compound and little fluorescence can be detected. Later, actively growing organisms consume the oxygen and allow the fluorescence to be detected.</p> <p>Tubes are monitored by the BACTEC 960 every 60 minutes for increasing fluorescence. Analysis of the fluorescence is used to determine if the tube is positive.</p>
When would this media be used?	MGIT tubes are inoculated each day that clinical specimens are received in the lab. After undergoing the decontamination/concentration process, all specimens received for routine culture are inoculated onto this media.
How long before growth is obtained?	<p>For mycobacteria, from 1 week to 6 weeks.</p> <p>Negative (no growth) MGITs are held for 6 weeks before reporting as negative.</p>
How are the results classified?	Positive, Negative, or Contaminated
What do the results mean?	<p>Positive: Growth noted. Identification procedures are started.</p> <p>Negative: No growth.</p> <p>Contaminated: Growth other than mycobacteria is present.</p>
Are other tests needed?	The TB Lab will initiate identification testing if the MGIT is positive. Susceptibilities will be ordered by the lab when indicated.
How would this test be ordered?	NA
How much would this test cost?	The GA Public Health Laboratory does not charge the county or the district for this procedure.

Amplified Mycobacterium Tuberculosis Direct Test (MTD)

How does it work?	<p>The Amplified Mycobacterium tuberculosis Direct Test (MTD) is a target-amplified nucleic acid probe test for the detection of <i>M. tuberculosis</i> complex rRNA in concentrated specimen sediments prepared from sputum, bronchial specimens (BAL or bronchial aspirates), or tracheal aspirates.</p> <p>The MTD test is intended for use only with specimens from patients showing signs and symptoms consistent with active pulmonary tuberculosis. Patients who have received no antituberculous therapy, or less than 7 days of such therapy, or have not received such therapy in the last 12 months may be evaluated with this test.</p> <p>MTD testing does not take the place of culture. A negative MTD test does not exclude the possibility of isolating <i>M. tuberculosis</i> from culture.</p>
When is this test run?	The Georgia Public Health Laboratory will reinstate MTD testing January 1, 2005. Test days will vary.
How long before results are ready?	Results are available the same day the test is run.
How are the results classified?	Positive, Negative, and Indeterminate.
What do the results mean?	<p>Positive: <i>M. tuberculosis</i> complex rRNA is detected.</p> <p>Negative: <i>M. tuberculosis</i> complex rRNA is not detected.</p> <p>Indeterminate: Equivocal results. Test must be repeated.</p>
Are other tests needed?	A culture for <i>M. tuberculosis</i> must be performed.
When would this test be used?	This test would be ordered on a specimen from a patient showing signs and symptoms consistent with active pulmonary tuberculosis to see if <i>M. tuberculosis</i> complex rRNA is present in the specimen.
How would this test be ordered?	By submitting a MTD Request Requisition to the GPHL or by calling the TB Laboratory and requesting the test.
How much would this test cost?	The GA Public Health Laboratory does not charge the county or the district for this test.

Polymerase Chain Reaction (PCR)	
How does it work?	<p>Polymerase Chain Reaction (PCR) is a sensitive laboratory technique that is used to detect and repeatedly copy small amounts of DNA or RNA.</p> <p>Once the PCR product has been formed, it can be used in further applications.</p>
When is this test run?	<p>Currently, this testing technique is not performed by the State TB Lab.</p> <p>Testing using this molecular technique is slated to begin January 1, 2005.</p>
How long before results are ready?	NA
How are the results classified?	NA
What do the results mean?	NA
Are other tests needed?	NA
When would this test be used?	NA
How would this test be ordered?	NA
How much would this test cost?	NA

Restriction Fragment Length Polymorphism (RFLP)

How does it work?	Restriction Fragment Length Polymorphism (RFLP) is a molecular technique in which organisms may be differentiated by analysis of patterns derived from cleavage of their DNA. If two organisms differ in the distance between sites of cleavage of a particular restriction endonuclease, the length of the fragments produced will differ when the DNA is digested with a restriction enzyme. The similarity of the patterns generated can be used to differentiate species of organisms from one another.
When is this test run?	The State Lab does not perform this test. Isolates are sent to the California State Genotyping Lab for analysis.
How long before results are ready?	Results are usually available 3 to 4 weeks after the California Genotyping Lab receives the isolate.
How are the results classified?	NA
What do the results mean?	The results show a mapping of the restriction fragment.
Are other tests needed?	No
When would this test be used?	<ul style="list-style-type: none">• To rule out cross-contamination of specimens.• To determine if two or more patients are infected with strains of <i>M. tuberculosis</i> possessing identical genotypes.• To determine reactivation vs. reinfection of a patient.
How would this test be ordered?	Physicians can request this test by calling the Georgia Public Health TB Lab.
How much would this test cost?	There is no charge for this test.

MTB Susceptibility Testing	
How does it work?	<p>M. tuberculosis isolates are tested for sensitivity/resistance to isoniazid, rifampin, and ethambutol using the BACTEC MGIT 960 susceptibility method. If resistance is detected in any of the three drugs, the susceptibility is repeated and streptomycin is added to the drug panel. All isolates showing resistance are submitted to CDC for confirmation.</p> <ul style="list-style-type: none"> A standardized suspension of MTB is added to 4 MGIT tubes. Tube 1 is a growth control (contains no antibiotics), Tube 2 contains isoniazid, Tube 3 contains rifampin, and Tube 4 contains ethambutol. The MGIT tubes are placed in the BACTEC 960 which scans the tubes for growth once every hour. When the growth control tube has reached 400 growth units, the BACTEC 960 performs a final scan on tubes 2, 3, and 4 to check for growth and then prints out a final report. Based on growth units, it can be determined if the MTB isolate is sensitive or resistant to each of the drugs tested. If the MTB is sensitive to a drug, there would be no growth in that MGIT tube. If the MTB is resistant to a drug, then the MTB would grow in the MGIT tube and the growth units would be registered on the BACTEC 960 report.
When is this test run?	Wednesdays and Fridays
How long before results are ready?	4 to 13 days after the actual susceptibility testing is started. Isolates must meet certain criteria before susceptibility testing can be performed. This can add up to 7 days to the turn-around-time for the susceptibility results.
How are the results classified?	Sensitive, Resistant, or Contaminated
What do the results mean?	<p>Sensitive: The drug can be used to treat the MTB isolate. Resistant: The drug has no effect on MTB isolate. Contaminated: The susceptibility is contaminated with an organism other than MTB and will have to be repeated.</p>
Are other tests needed?	No, unless multi-drug resistance is demonstrated. Then the isolate is sent to CDC for an expanded panel of drug testing.
When would this test be used?	<ul style="list-style-type: none"> To determine the susceptibility of an MTB isolate to isoniazid, rifampin, ethambutol, and in some cases to streptomycin. When a patient is not responding to treatment and the physician feels that the susceptibility should be checked again. Every 3 months for as long as a patient's cultures remain MTB positive.
How would this test be ordered?	Through the State TB Lab. Susceptibilities are automatically run on all first-time MTB positive cultures.
How much would this test cost?	The GA Public Health Laboratory does not charge the county or the district for this test.

Clinical Algorithm applying acid- fast staining, MTD testing & Culture

(MTD testing is currently only approved for smear positive respiratory specimens.

It has not been validated in non-respiratory specimens.)

